

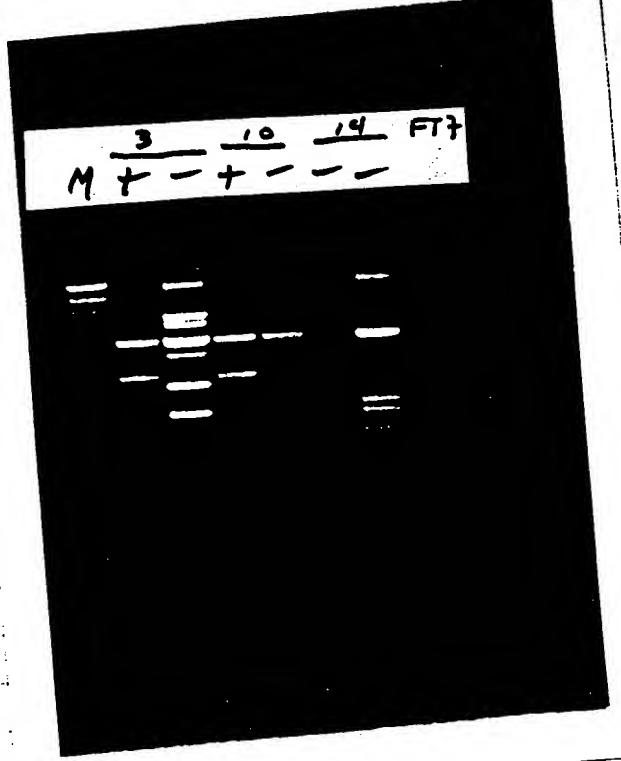
~~Exhibit~~ 3

2/2 Digest FT7 DNA 3, 10, 14 w/ Kpn I / Hind III
use previous prep as control (-)

Expected Size
(3) Same (4) Inters. (-)
3159 3159
1573 1366
625 782

10 3159 3159
1573 1121
650 832
380 650

14 3159 3159
1573 945
650 832
204 650



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Major problem w/ #14

Some how samples got mixed-up

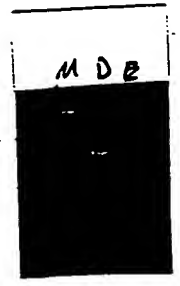
Go back & check out - Start for single colonies
A D: E

Also mini prep from original culture
Digest w/ Kpn I / Hind III 0/14

Also Run gel of Digests
Clearly Sample A which was given as mate prep is
in the wrong orientation

Start 0/14 of D: E to mini prep before
start of 500ml culture

That mini prep FT7 DNA 14-D: E
Digest w/ Kpn I / Hind III
run on gel - Both are fine
use -D for mate



7/25 Plasmid isolation of FT7, cDNA 14 - D up to Banding
 - do 500 nls in 2 250 sets, end w/ 2 tubes to Band
 7/26 Pull Bands - double band 1 pop - 6 hrs during the day

7/27 Digest FT7, cDNA 14 w/ Kpn I / Hae III
 ① - single bandy
 ② Double Bandy
 ③ FT7, cDNA 14 antisense



Check Absorbance / Concentration of

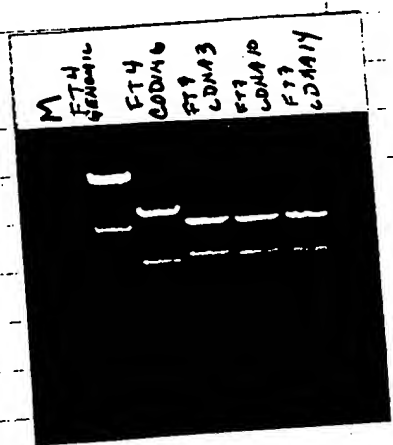
Samples	260	FT7 cDNA 14	280
①	.127	.062	.63 ng/μl 2.0
②	.143	.065	.71 ng/μl 2.3

Sequence
 FT7, cDNA 3
 T7
 cDNA 14
 T7
 8850
 9007
 9874

Digest Mouse FT4

	260	280	260/280
Pst I genomic	.085	.045	
Not cut	.106	.060	

Digest of cDNA 3
 Kpn I cDNA 10
 Hae III cDNA 14



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8/4 FACS Analysis of Transfected cells w/ following vectors

pCDNA 7

FT7, 1, 2a, 2b, 3

1, 2b, 3

1, 3

cDNA 3

cDNA 10

cDNA 14

2 plates / Vector - Divide

FACS 7mL

Yuko 5mL

EAT 3mL

ETassy 5mL

(7.5ul)

(100ul)

Antibodies

IgM H - black 1:100

IgM hrf green 1:1000

IgM SLx 1:200

IgG ha red 1:500

IgG SLa blue 1:500

2nd Antibody

IgM 2.5mL

12.5 / 2.5mL

IgG 1.5

60 / 1.5mL

Results are

H - all neg

hrf - all neg

SLx, pCD (-), 1, 2a, 2b, 3(+), 1, 2b, 3(+), 1, 3 (-), cDNA 3 (-), 10 (+), 14 (+)

ha all neg

SLa all neg

8/8 Symp 12 pum KG FT4

6451

6080

2470

6199

6374

6087

6306

6086

6203

6085

5721

5671

8/9 Run sy gel of above samples

Also sy

5728

6084

7213

5731

5737

6082

5662

5727

6201

6200

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8/10 Sequencing gel of 8/9 samples
Sequence

FT4 6079
6202
6307
6373
CDNA10 715
946

CDNA 14
T7
8850
8807
8874

Protein assay of FACS Samples, Also CAT assay

pcDNA

FT7-1,2a2b,3

FT7,1,2b,3

FT7,1,3

CDNA3

CDNA10

CDNA14

BSA Blank Protein

0 1.00 116 108

1 0.20 225 214

2 0.36 383 376

4 0.69 673 682

8 1.21 1230 1222

16

Sample

pcDNA 0.292 0.294 0.293

1,2a2b,3 0.337 0.330 0.33

1,2b,3 0.298 0.343 0.32

1,3 0.369 0.372 0.37

CDNA3 0.363 0.379 0.37

CDNA10 0.306 0.298 0.302

CDNA14 0.225 0.253 0.239

FACS Results

Only stain w/ shx

1,2a2b,3 23.6%

1,2b,3 24.6%

CDNA10 14.9%

CDNA14 8.0%

Micro BCA Protein Assay

Reagent mic	MC	MB	MA
Per assay tube (ml)	0.01	0.24	0.25
Cocktail for Tubes			

Incubate 1 h at 60°C and cool to room temp.
Since the color development has no end point, all tubes must be heated and cooled at the same time

1 mg/ml BSA (l)	Water (l)	Reagent (l)	Abs. 562	
0.0	500.0	500.0	Blank	Slope = 0.0734 Y intercept = 0.0656 X intercept = -0.8940 R = 0.9985
1.0	499.0	500.0	0.108	
2.0	498.0	500.0	0.214	
4.0	496.0	500.0	0.376	
8.0	492.0	500.0	0.682	
16.0	484.0	500.0	1.222	

8/10 Sequencing of FT4 9/10 Samples

Sample	l in assay	Water (l)	Reagent (l)	Abs. 562	mg protein/ml
pcDNA1	5.00	495.00	500	0.293	0.62
FT7 1,2a2b,3	5.00	495.00	500	0.333	0.73
FT7 1,2b,3	5.00	495.00	500	0.320	0.69
FT7 1,3	5.00	495.00	500	0.370	0.83
cDNA 3	5.00	495.00	500	0.371	0.83
cDNA 10	5.00	495.00	500	0.302	0.64
cDNA 14	5.00	495.00	500	0.239	0.47

CDMA 1X

$$H_2O \quad \dots \quad \underline{300}$$

SD / tube

CAT

Vector	Counts/5ul	8/12/94	Incorporated Counts (.85)	Total Counts	Total Counts Incorporated			
pcDNA1	11,349	11,829	9,189	8,063	238,189	244,643	9,673	8,487
FTT 1,2a2b,3	11,181	11,441	27,211	21,919	250,431	250,739	28,643	23,073
FTT 1,2b,3	11,772	11,826	37,541	40,684	272,981	277,204	39,517	42,825
FTT 1,3	11,215	11,690	23,076	28,706	247,376	262,506	24,291	30,217
cDNA 3	11,834	11,206	33,885	39,096	270,565	263,216	35,668	41,154
cDNA 10	12,017	11,312	30,068	33,165	270,408	259,405	31,648	34,911
cDNA14	11,079	11,570	44,133	40,529	265,713	271,929	46,456	42,662
Control		10,354		424		207,504		446
	Protein Conc. (ug/2.5ul)	Total Counts Inc- Bkg	% INC/hr	% INC/hr/ug			Mean CAT Activity	
pcDNA1	1.55	9,249	8,063	3.92	3.30	2.53	2.13	2.33
FTT 1,2a2b,3	1.83	28,219	22,649	11.27	9.03	6.17	4.95	5.56
FTT 1,2b,3	1.72	39,093	42,401	14.32	15.30	8.33	8.89	8.61
FTT 1,3	2.07	23,867	29,793	9.65	11.35	4.66	5.48	5.07
cDNA 3	2.07	35,244	40,730	13.03	15.47	6.29	7.48	6.88
cDNA 10	1.60	31,224	34,487	11.55	13.29	7.22	8.31	7.76
cDNA14	1.17	46,032	42,238	17.32	15.53	14.81	13.28	14.04

Q. Sample Data of FT7 FACS, CAT assay / give to Judo
Work on FT4 source

95 7 days sequencing on TruSeq PE4 samples

6451	0	6200
6378		6079
6306		6307
6203		1897
5721		1898
		1899

9/10 Sequencing gel of 8/15 samples (F.F.9) Formamide gel
 Prove Dr. Davis' reaction But w/ GAP prove
 To check condition of RFL

9/12 The F diagra technique didn't resolve all of the compressions
Try a terminal transposase technique
Run standard symenase rxn, after extension made
Heat tubes (A, C, G, T-) for 1.5 min 100°C
Hold on ice 10 min, Purpurn TdT/ONTF cocktail
Add to tubes, 37°C 30 min
Add Spl Stop